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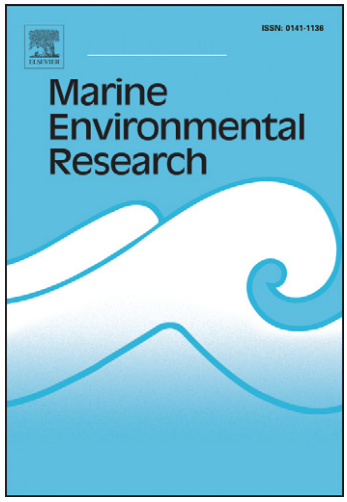

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PII: S0141-1136(08)00043-3
DOI: 10.1016/j.marenvres.2008.02.038
Reference: MERE 3195

To appear in: *Marine Environmental Research*

Please cite this article as: Denier, X., Couteau, J., Baudrimont, M., Hill, E.M., Rotchell, J., Minier, C., In vitro study of the effects of cadmium on the activation of the estrogen response element using the YES screen, *Marine Environmental Research* (2008), doi: 10.1016/j.marenvres.2008.02.038

In vitro study of the effects of cadmium on the activation of the estrogen response element using the YES screen

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Abstract

Estrogenic potential of environmental samples is frequently assessed using receptor-based functional assays. Using the Yeast Estrogen Screen (YES) developed by Routledge and Sumpter, we assessed the ability of cadmium to activate the estrogen receptor-mediated response. No induced transcriptional activity was observed with a range of CdCl2 concentrations (1nm-1mM). But, when combining cadmium with the model compound 17β-estradiol, cadmium was able to significantly potentiate the induced estrogenic response for concentrations ranging from 15 nM to 1 µM. A maximal effect was observed at 0.5 µM with a ten fold reduction of the 17βestradiol EC50.

Keywords: Cadmium; Yeast; Estradiol; Estrogen-receptor; Endocrine disruption

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Important efforts have been made for the development of screening strategies to evaluate and understand endocrine disruption by estrogenic compounds in human and wildlife. Various *in vitro* tests have been developed and are used to assess the estrogenicity of isolated compounds or complex environmental mixtures. One of the most widely used *in vitro* tests is the yeast estrogen screen (YES; Routledge and Sumpter, 1996).

Metals, and particularly cadmium, have already been shown to act *in vivo* as endocrine disruptors. But action through a direct estrogenic pathway is still unclear. Several studies have found an *in vitro* estrogenic activity of cadmium. Cadmium induced growth in the human breast cancer cell line MCF-7 (Garcia-Morales et al., 1994, Choe et al, 2003). Stoica et al. (2000) showed that cadmium is able to activate the estrogen receptor by binding to the hormone binding domain of the receptor. Choe et al. (2003) and Wilson et al. (2004) also observed induction of an estrogen receptor-dependent transcriptional expression assay (MCF-7-ERE or TD47 cell lines).

But contradictory results have been reported. Le Guevel et al. (2000) used yeast cells transfected with rainbow trout estradiol receptor and observed that cadmium alone is unable to activate the receptor. Silva et al. (2006) using the YES and E-screen assays (MCF-7 proliferation) concluded a lack of activity of cadmium in both tests. In view of these contradictory results, this study aimed to assess the estrogenicity of a wide range of cadmium concentrations and to evaluate its potential to modulate the 17-β-estradiol activity in the YES assay.

$CdCl_2$ was obtained from Sigma-Aldrich. From 10mM $CdCl_2$ stock solution, 21 different concentrations ranging from 1 mM to 1 nM were obtained by serial dilution in water

then sterilized by 0.22 µm filtration. Yeast estrogen screen was conducted according to Routledge and Sumpter (1996). A serial dilution of estradiol-17β in ethanol was used as reference for the test. 10 µl of each dilution were added to wells. "Metal alone" and blank wells received 10 µl ethanol instead. 20 µl of cadmium solutions were then added to wells, while "estradiol alone" and blank wells received 20 µl water. Finally, 180 µl of assay medium and yeast were added to the wells. Plates were incubated 48 hours at 30°C before absorbance reading. In the yeast estrogen screen, the activation of estrogen receptor leads to its binding to estrogen response element then subsequent synthesis of β-galactosidase. The galactosidase hydrolyses the chlorophenol red-galactopyranoside present in the medium. The chlorophenol-red produced was measured by absorbance at 540nm. Absorbance at 620 nm allows correction for turbidity: corrected absorbance = Abs_{540nm} - (Abs_{620nm} –Abs_{620nm} blank).

We first tested the ability of $CdCl_2$ to directly activate the ER-mediated response in the YES assay. Fig. 1 indicates that none of the tested cadmium concentrations resulted in any significant galactosidase activity as previously reported in other studies using yeast cells (Le Guevel et al., 2000; Silva et al., 2006). Although the human estrogen receptor and the estrogen response element have been inserted in the yeast strain, it appears that the YES assay can generate results that are different from those obtained with mammalian cell lines. Indeed several studies have reported that cadmium can activate the human reporter by interacting with the ligand-binding domain and exerts effects characteristic of the endogenous hormone in human cells (Stoica et al., 2000; Choe et al., 2003; Wilson et al., 2004).

In a second experiment, we assessed the putative joint action of cadmium and the model compound 17βestradiol (E2). 21 different concentrations (from 1nM to 1 mM) of cadmium were tested in combination with 11 E2 concentrations (10pM to 10nM). Results are shown in Fig. 2. Different effects were observed according to the cadmium concentration in the test medium. Cadmium concentrations from 1 to 500 nM potentiated the estrogenic

activity in a dose-dependant manner (although significant differences were only obtained for concentrations of 15 nM $CdCl_2$ and higher). For each curve, the maximum induction was not affected but the EC_{50} decreased. Maximum effect (EC_{50} reduced to one tenth of the control value) was observed with 0.5 µM $CdCl_2$.

Cadmium concentrations from 1 to 20 µM reduced the enzymatic activity in a dose dependent manner. The decreased activity appeared to be, at least, partly due to toxicity as indicated by the reduced absorbance at 620nm (not shown). The curve obtained with 2 µM $CdCl_2$ did not differ significantly to the standard curved obtained with E2 alone but a rapid decrease in galactosidase activity was observed for higher concentrations. Finally, at cadmium concentrations from 30 µM to 1 mM $CdCl_2$, yeast growth was inhibited.

These results show that cadmium can either potentiate the estrogenic response or have toxic effects on yeast metabolism. Reports from Silva et al. (2006) and Le Guevel et al. (2000) did not show potentiating effect of cadmium on their yeast-based assay but significant decrease in the response possibly due to toxicity. It should be stressed that only a few cadmium concentrations together with E2 were tested in these studies. Le Guevel and colleagues incubated the cells in 10 µM cadmium only, a concentration which was already toxic in this study. Cadmium toxicity at 10 µM or higher concentrations of cadmium has recently been reported either in the YES or in the E-screen assays.

The reason why cadmium cannot directly activate the response mediated by the human estrogen receptor in the YES assay is unclear. But results obtained in this study indicate that cadmium can potentiate the estrogenic response induced by the interaction of E2 with the ER. This effect was not due to an enhanced metabolic activity as cadmium did not significantly increase yeast growth at effective concentrations (data not shown). Predki and Sarkar (1994) showed that cadmium, copper and chromium can interact with the zinc finger DNA-binding domain of the ER and activate gene transcription. These effects may have also occurred in our

study and account for the observed response. In conclusion, these results add further evidence that cadmium may act as potent endocrine disrupter and may increase the effect of hormonally-active compounds.

Acknowledgments

This work is part of the European Interreg III program "Risk Analysis associated with Endocrine Disruption in the Manche Regions (RAED)".

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Figure Captions

Fig. 1. Effect of 17β-estradiol (E2) and cadmium in the recombinant yeast estrogen screen (YES).

Fig. 2. Variation of yeast estrogen screen response to 17β-estradiol with increasing concentrations of cadmium. The standard curve obtained with 17β-estradiol (E2) alone is indicated with the error bars (n=14). Added cadmium concentrations are indicated in the table for each numbered curves. Plain lines indicate a decreased E2 EC50, dotted lines indicate an increased E2 EC50 with increasing cadmium concentrations. Curves 2, 3, 4, 6 and 7 are not displayed to gain clarity. Curves with E2 + cadmium were derived from two independent experiments.

Figure 1

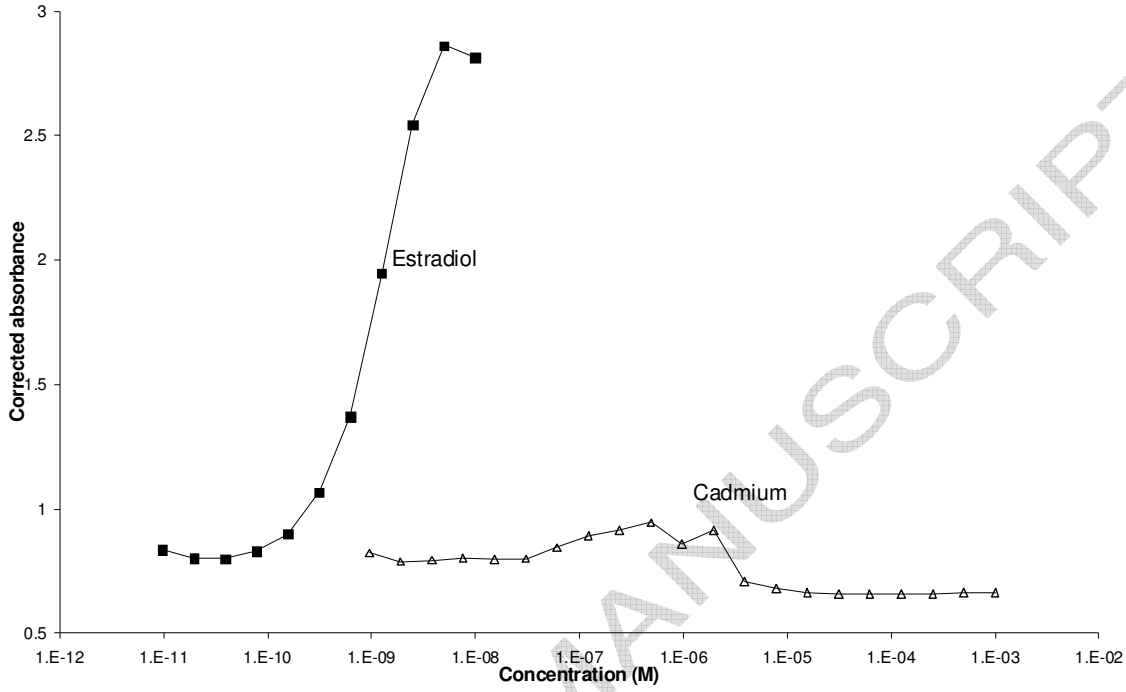

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Figure 2